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# The Drosophila *couch potato* Gene: An Essential Gene Required for Normal Adult Behavior

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ABSTRACT

Through enhancer detection screens we have isolated 14 insertions in an essential gene that is expressed in embryonic sensory mother cells (SMC), in most cells of the mature embryonic peripheral nervous system (PNS), and in glial cells of the PNS and the central nervous system (CNS). Embryos homozygote for amorphic alleles die, but show no obvious defects in their cuticle, PNS or CNS. The gene has been named *couch potato* (*cpo*) because several insertional alleles alter adult behavior. Homozygous hypomorphic *cpo* flies recover slowly from ether anaesthesia, show aberrant flight behavior, fail to move toward light and do not exhibit normal negative geotactic behavior. However, the flies are able to groom and walk, and some are able to fly when prodded, indicating that not all processes required for behavior are severely affected. A molecular analysis shows that the 14 insertions are confined to a few hundred nucleotides which probably contain key regulatory sequences of the gene. The orientation of these insertions and their position within this DNA fragment play an important role in the couch potato phenotype. *In situ* hybridization to whole mount embryos suggest that some insertions affect the levels of transcription of *cpo* in most cells in which it is expressed.

CELLS of the nervous system require the action of many genes to acquire morphological and functional identity. A number of genes involved in nervous system development and function have been identified and partially characterized in Drosophila (for reviews see Campos-Ortega and Knust 1990; Jan and Jan 1990; Ghysen and Dambly-Chaudiere 1989; Rubin 1988; Ganetzky and Wu 1986; Hall 1985). Genetic screens for mutants that affect the nervous system have typically focussed on isolating mutants that are either defective in their development, exhibiting morphological aberrant structures (*e.g.*, *Notch*, *scute*, *achaete*, *slit*, *sim*, etc.) or mutants that display quite specific behavioral defects in adults (*e.g.*, *Shaker*, *rutabaga*, *per*), often exhibiting subtle physiological or biochemical alterations.

The advent of enhancer detection (O'Kane and Gehring 1987; Bellen *et al.* 1989; Bier *et al.* 1989) provides new means to study genes that are expressed and/or required in the nervous system (Mlodzik *et al.* 1990; Doe *et al.* 1991; for review see Bellen, Wilson and Gehring 1990). We have screened for enhancer detector insertion strains that express the β-galactosidase gene in the sensory mother cells (SMC) of the peripheral nervous system (PNS). The goal of these studies is to identify genes which play an important role in the differentiation and/or function of the PNS and to further unravel the genetic cascade which leads to a fully mature PNS. Here, we describe the isolation of an allelic series of a newly isolated gene which is expressed in the SMC, the embryonic and adult mutant phenotypes, the molecular defects that underlie some of these mutations, and the cloning of the gene.

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## MATERIALS AND METHODS

**Fly stocks:** Generation of flies that carry enhancer detectors is described in Bellen *et al.* (1989), and Bier *et al.* (1989). Some of the insertion strains described in these papers were renamed. The *cp1* insertion strain corresponds to the P[lArB] B52.1M3 strain described in Bellen *et al.* (1989) and Wilson *et al.* (1989). The *l3* insertion corresponds to l(3)5D4 in Bier *et al.* (1989). Most strains with 90D/E deficiencies were obtained from Kevin Moses (Moses, Ellis and Rubin 1989), except *Df(3R)DG4* which was obtained from Don Gailey (Gailey and Hall 1989) and *Df(3R)P14*, which was obtained from the Bowling Green Drosophila stock center (Ohio) and the Umea Drosophila stock center (Sweden).

The *lΔcp11* and *lΔcp12* alleles were recovered by remobilizing P[lArB], after isogenization, as described in Bellen *et al.* (1989).

***In situ* hybridization and immunocytochemistry:** *In situ* hybridizations to whole-mount embryos were performed according to a modified protocol of Tautz and Pfeifle

(1989). Probes were labeled with digoxigenin-dUTP and hybridization was detected with a monoclonal antibody against digoxigenin coupled to alkaline phosphatase (Boehringer Mannheim). *In situ* hybridization to polytene chromosomes was performed according to a modification of the protocol described in LANGER-SAFER, LEVINE and WARD (1982). This protocol was adapted for digoxigenin-labeled probes.

$\beta$-Galactosidase activity in embryos was detected as described in BELLEN *et al.* (1989). $\beta$-Galactosidase protein and other proteins were localized immunohistochemically using a polyclonal rabbit anti-$\beta$-galactosidase antibody (Cappel) or monoclonal antibodies (see text) as described in BIER *et al.* (1989).

**Phototaxis assay:** Phototactic tests were performed in a seven-trial countercurrent apparatus as described by BENZER (1967). After a 1-min equilibration period, groups of 20–25 flies were given 1 min to move toward a white light source in an otherwise dark room. At the end of the assay, flies in each tube were counted, and the number of flies in each tube was multiplied with the number of times that they moved toward the light. The sum of these values was divided by the total number of flies. The theoretical range is thus 0–7 (0 = no flies move toward light, 7 = all flies move 7 times toward light). Wild-type flies, *e.g.* Canton-S, typically score higher than 4. Each assay was performed at least three times. (+) = index $\geq$ 4, (+/−) = 3 $\leq$ index < 4, (−) = index < 3, nt = not tested.

**Flight response assay:** Groups of 20 flies were given 30 sec to fly away from a empty Petri dish or a 95 × 28 mm vial in which they were kept for at least 1 hr after anaesthesia. The assays were repeated at least three times. (+) = 61–100%, (+/−) = 31–60%, (−) = 0–30% fly away.

**Geotaxis assay:** About 20 flies were transferred to an empty 95 × 28 mm vial which was subsequently capped with another empty vial. Flies were shaken to the bottom of one of the vials in a dark room and the number of flies that move beyond the top of the bottom vial in 15 sec were counted. The assays were repeated at least three times. (+) = 61–100%, (+/−) = 31–60%, (−) = 0–31% move up, nt = not tested.

**Ether recovery assay:** About 20 flies were anaesthetized for 1 min in an etherizer containing 2 ml of ether. The flies were then placed in a 100-mm Petri dish and the recovery time (RT) was recorded when 50% of the flies walk away or start grooming ($RT_{50}$), and when all flies recovered ($RT_{100}$). Fifty percent of wild-type flies (Canton-S) will typically recover in less than 15 min, 100% will recover in less than 25 min. (+) = $RT_{50}$ <15 min, $RT_{100}$ < 25 min, (+/−) = 15 min < $RT_{50}$ < 20 min, 25 min < $RT_{100}$ < 30 min, (−) = 20 min < $RT_{50}$, 30 min < $RT_{100}$.

**Recombinant DNA:** To recover DNA sequences flanking the enhancer detectors we used the plasmid rescue techniques described in WILSON *et al.* (1989) and BIER *et al.* (1989). Genomic fragments were recovered from three different strains. Some of these fragments were then used to screen a lambda DASH genomic library (kindly provided by RON DAVIS). Maps were constructed with the *Eco*R1, *Hind*III and *Xba*I restriction enzymes.

## RESULTS

**Screening for enhancer detectors that express $\beta$-galactosidase in the PNS:** In an attempt to identify new genes that are expressed at the onset of nervous system development, embryos of 4,600 strains carrying single *P* element-enhancer detectors were stained for $\beta$-galactosidase activity (BIER *et al.* 1989; BELLEN *et al.* 1989; WILSON *et al.* 1989). Fourteen insertion strains that stain most or all precursor cells of the developing embryonic PNS were selected. Very few other strains identified in these screens express the *lacZ* gene in the SMC, suggesting that few specific genes may be active in these cells. This prompted us to further analyze these insertion strains. Surprisingly, 14 enhancer detector strains carry an insertion at cytological subdivision 90D. These observations indicated that the enhancer detectors are controlled by regulatory elements of the same gene, and that the 90D division is possibly the hottest spot for *P* element insertions as one in every 300 insertions maps at 90D (BIER *et al.*, 1989). Five insertion strains are homozygous viable, and nine strains are homozygous lethal.

In a separate screen for homozygous viable mutations that cause reduced flight abilities (A. KOLODKIN and COREY GOODMAN, personal communication), a *P* element-enhancer detector strain was isolated that maps at 90D. Embryos of this strain express $\beta$-galactosidase in essentially the same cells as the other 90D insertion strains.

**$\beta$-Galactosidase expression in enhancer detector strains with insertions at 90D:** The *lacZ* staining pattern in embryos of the 14 90D strains is, with the exception of a number of midgut cells, confined to the nervous system. *lacZ* expression is first seen in stage 8 embryos in a few cells at the most anterior end of the embryo (Figure 1A) (for staging see CAMPOS-ORTEGA and HARTENSTEIN 1985). Next, we observe staining in the SMC and differentiating cells of the PNS in stage 9–11 embryos (see Figure 1, B, C and D). The pattern consists of a gradual increase from one cell in each presumptive segment to a set of four two-cell clusters. During extended germ band (stage 11) the pattern rapidly becomes more complex. In subsequent stages all known PNS neurons as well as their sibling non-neuronal support cells express the $\beta$-galactosidase (Figure 1, E and F) (see also Figure 6, B and C, in BIER *et al.* 1989). *lacZ* expression in the PNS of the thoracic and abdominal segments resembles the expression pattern observed in the A37 enhancer detector strain (see GHYSEN and O'KANE 1989). This latter enhancer detector maps at 80A.

The cells and organs of the PNS in the head region of the embryo have been described in less detail than those of the thoracic and abdominal segments (CAMPOS-ORTEGA and HARTENSTEIN 1985; JURGENS *et al.* 1986), making assignments of particular clusters of cells that are labeled in this region more difficult. The number of cell clusters expressing *lacZ* in the gnathal segments and the procephalon gradually increase from one during late gastrulation (stage 7) to 13 during early head involution (stage 14; see Figure 2). Based on the topological position of these clusters in

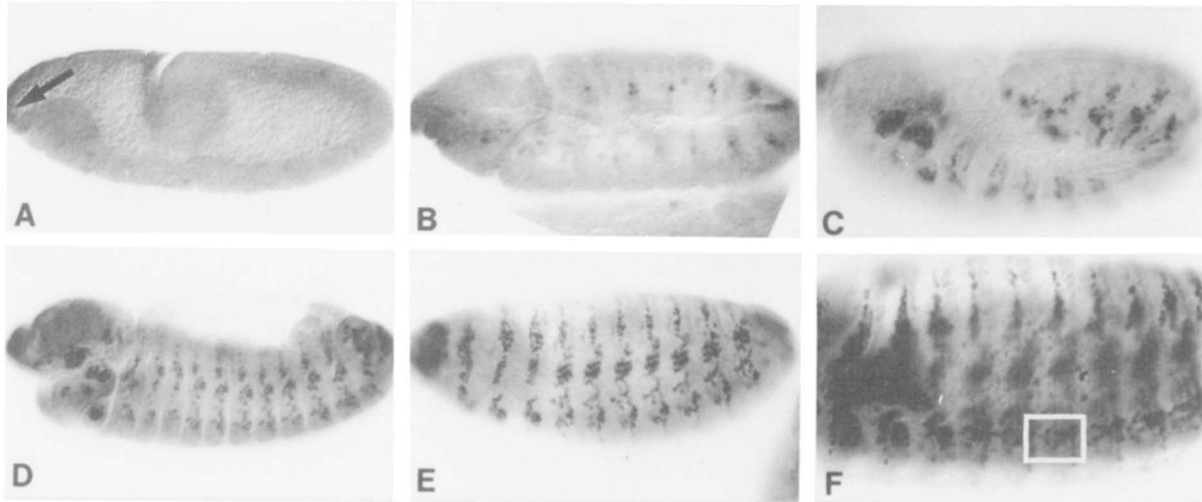


FIGURE 1.—β-Galactosidase expression pattern in homozygous $cpo^{cp1}$ embryos at various stages of development. All embryos are shown with their anterior end to the left, and their dorsal side up. A, Stage 8 embryo, *lacZ* is only weakly expressed at the most anterior end of the embryo (see arrow). B, Stage 10 embryo, *lacZ* expression is confined to one or two cells per segment. C, Stage 12 embryo, the *lacZ* expression pattern is already quite complex and relatively large clusters of cells in each segment are labeled. D, Stage 13 embryo, note the staining in the head region as well as the regular pattern in PNS of abdominal and thoracic segments. E, Stage 16 embryo, all the cells except some of the support cells of a few external sensory organs of the PNS express β-galactosidase. F, Detailed view of a stage 16 embryo. Focus is on the CNS and most of the cells of the PNS are out of focus. The CNS is at the bottom of the embryo. Note the glial cells that are located along the dorsal side of the CNS (see rectangle).

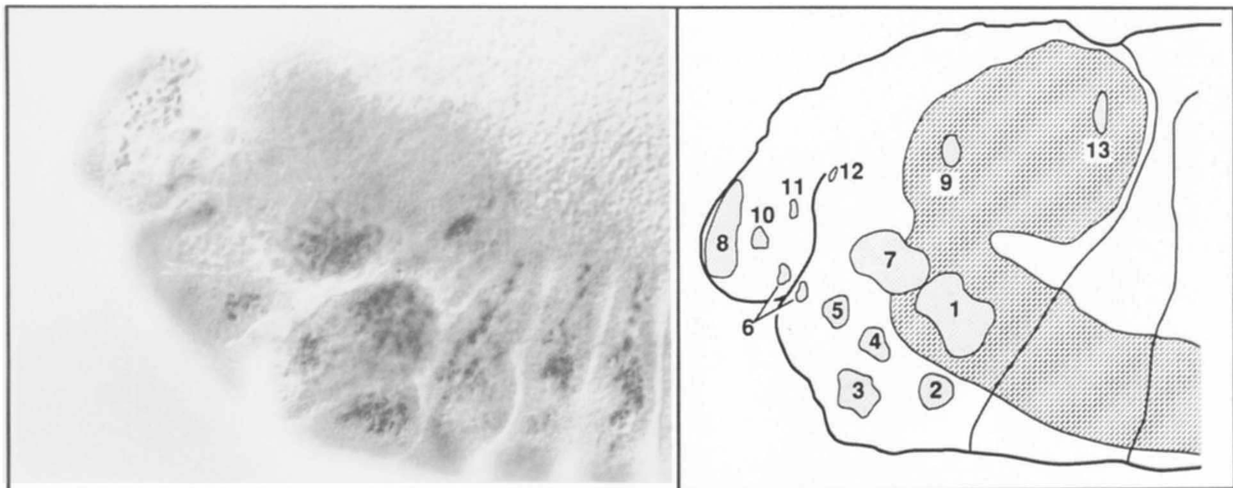


FIGURE 2.—Detail of the β-galactosidase expression pattern in the head region before head involution (stage 14). A, Lateral view of the cephalic region of an $cpo^{cp1}$ embryo stained immunocytochemically with an anti-β-galactosidase antibody just before head involution. B, Composite and schematic drawing of a lateral view of the cephalic region of an embryo, prior to head involution. This drawing is based on observations made on several embryos and does not correspond exactly to the picture shown on the left. The clusters that are labeled with anti-β-galactosidase antibody are numbered and the CNS in the background is hatched. For simplicity we have chosen the nomenclature used by JURGENS *et al.* (1986). 1, Central group of maxillary sense organ. 2, Lower lip organ. 3, Labial sense organ. 4, Ventral organ. 5, Dorsal lateral papilla of maxillary sense organ. 6, Hypopharyngeal organ. 7, Antennal sense organ. 8, Labral sense organ. 9, Dorso-medial papilla of maxillary sense organ. 10–13, Clusters of cells that probably belong to the PNS of the head and have not been described previously to our knowledge.

stage 14 embryos, we believe that nine of these 13 clusters have been described previously (for a comprehensive summary see Table 7 in JURGENS *et al.* 1986). The other four clusters have not been described previously perhaps because they are quite small and consist of 2–5 cells compared with 10–40 cells for most of the other clusters. Because the cells of these clusters are epidermal and subepidermal, and most of the staining cells are confined to cells of the PNS, we believe that these cell clusters also belong to the PNS.

In addition to the neurons and support cells of the PNS, *lacZ* expression is also observed in some cells along the dorsal and ventral area of the ventral nerve cord of the CNS, in cells along the base of the bilateral peripheral nerve roots, and in cells laterally along the anterior fascicle of the peripheral nerves. β-Galactosidase expression can clearly be seen in these cells after the completion of germ band retraction (stage

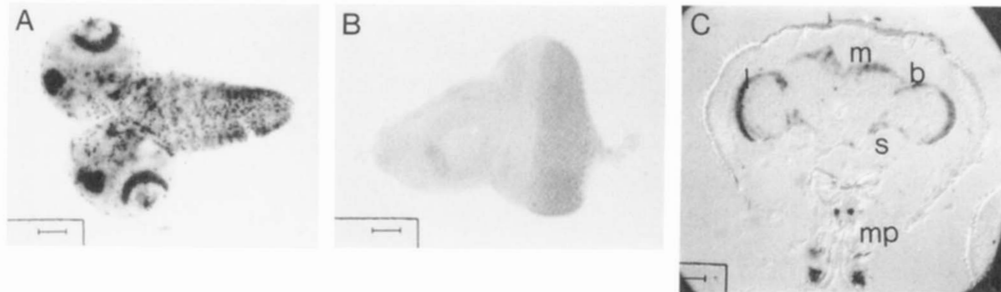


FIGURE 3.—β-Galactosidase expression pattern in larval brain, eye imaginal disc and adult head of the *cp1* insertion strain. A, Third instar larval brain. Note the expression in four main clusters of cells. The two central clusters correspond in position and size to the cell bodies of the neurons of the mushroom bodies. The two lateral moonshaped clusters correspond to the area where the photoreceptor cells of the eye imaginal disc synapse with neurons of the brain. Many cells scattered throughout the nervous system also express *lacZ*. B, Eye-antennal imaginal disc. Note the staining behind the morphogenetic furrow. C, Section of adult head. All staining is nuclear: m, musroom bodies; l, lamina; b, brain rind; s, suboesophageal ganglion; mp, four clusters in mouth parts.

13). Based on their morphology and position, these cells probably correspond to glial cells, some of which have been described previously (JACOBS and GOODMAN 1989; JACOBS *et al.* 1989; KLAMBT and GOODMAN 1991; FREDIEU and MAHOWALD 1989).

β-Galactosidase expression was also examined in third instar larvae brains and imaginal discs. As shown in Figure 3A, *lacZ* is expressed in the larval brain in four main clusters of cells. One set has a moonshaped form in each hemisphere of the brain. These cells probably correspond to the cells with which the photoreceptor cells from the eye imaginal disc synapse in the brain. The second bilaterally symmetric cluster of cells corresponds in size and location with the cell bodies of the mushroom bodies. This structure of the brain is thought to play a key role in olfactory learning and memory. In addition to these four cell clusters many scattered cells in the brain also express *lacZ*. Finally, three clusters of cells which are associated with the brain and located between both cerebral hemispheres also express *lacZ* (not shown). One cluster is small, located centrally and ball shaped. The other two are larger, form a pair, and are pear shaped. These three clusters probably belong to the ring gland and/or the stomatogastric nervous system (CAMPOS-ORTEGA and HARTENSTEIN 1985).

β-Galactosidase expression in imaginal discs is, with the exception of the eye disc, confined to single cells or small to medium size clusters of cells. We compared the *lacZ* expression pattern in the wing imaginal disc of the *cp1* insertion strain with the pattern decribed by GHYSEN and O'KANE (1989) for the A37 enhancer detector which is expressed in the PNS. Both patterns are similar in the notum but the triple row bristles precursor cells in the wing region are not labeled in the *cp1* wing disc. In the eye imaginal disc many photoreceptor cells express β-galactosidase behind the morphogenetic furrow (see Figure 3B). Finally, *lacZ* expression is not restricted to the PNS and the CNS in third instar larvae since the salivary glands express β-galactosidase.

In the adult fly we only examined β-galactosidase expression in sections of the head. As shown in Figure 3C, staining is observed in the lamina, brain rind, suboesophageal ganglion and clusters of cells in the mouthparts. The *lacZ* is also expressed in many nuclei of the retina, medulla, lobula and lobular plate, ocelli and ocellar ganglion. Hence, most cells of the visual system express the marker gene. Many cells in the antennal segments also strongly express the marker gene. Furthermore, *lacZ* is expressed in many cells of the mushroom bodies and other parts of the brain. Finally, one or two cells stain at the base of many of the macrochaetae in the head. The marker gene is thus expressed in many cells of the adult PNS and CNS (PYUNG-LIM HAN, personal communication).

**Enhancer detector insertions at 90D affect an essential gene:** To map the lethal mutations (abbreviated *l1–l9*, l = lethal) on the chromosomes that carry the 90D insertions, we performed complementation tests with a series of deficiencies that lack cytological bands within or around the 90D/E subdivision. Some of the results of these complementation tests are shown in Figure 4 and Table 1. Lethal mutation(s) on all tested chromosomes that carry 90D/E insertions are uncovered by the deficiencies, *Df(3R)P-14*, *Df(3R)glBX10*, *Df(3R)l(3)BX6* and *Df(3R)DG4* (see MATERIAL AND METHODS), whereas *Df(3R)glBX-1* complements all the 90D insertion chromosomes. Moreover, the lethal insertion chromosomes (*l1-9* ) fail to complement each other. Hence, the insertions affect a common essential gene that maps to the 90D2-90F6 cytological bands (see Figure 4).

All the deficiencies that fail to complement the chromosomes containing a 90D insertion also fail to complement $sr^1$ (GAILEY and HALL 1989; see Figure 4). Homozygous $sr^1$ flies have a gray stripe on the thorax which is the result of the lack of the dorso longitudinal indirect flight muscle. Complementation tests with $sr^1$ and the 90D insertion chromosomes indicate that the essential gene at 90D complements

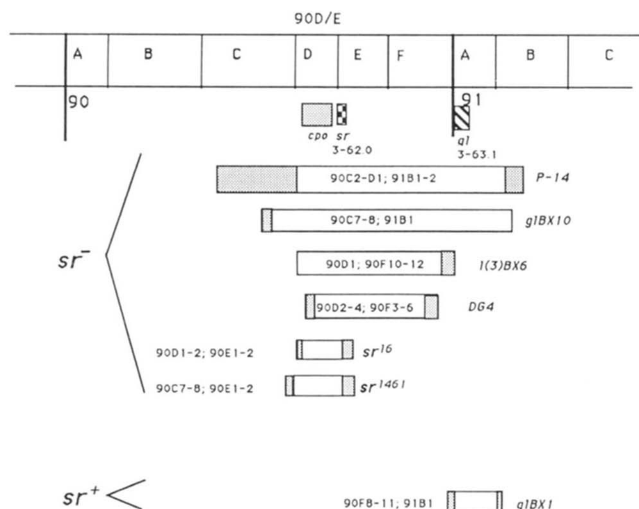


FIGURE 4.—Extent of some cytological visible deficiencies in the 90D/E region. The cytological extent of several deficiencies are shown. Some were provided by KEVIN MOSES (personal communication) or DON GAILEY (GAILEY and HALL 1989), and others were determined in this work ($Df(3R)sr^{l6}$, and $Df(3R)sr^{l461}$). Since there is no known breakpoint between *sr* and *cpo* the demarcation between the two genes is unknown.

the $sr^{1}$ allele as no gray stripe is observed in transheterozygous flies. However, $sr^{l6}$ and $sr^{l461}$, diepoxybutane (DEB)- and ethyl methanesulfonate (EMS)-induced alleles (DE LA POMPA, GARCIA and FERRUS 1989), fail to complement the lethality of the chromosomes that carry 90D insertions (see Table 1). Cytological examination of these *stripe* alleles reveals that they are small but cytologically visible deficiencies: $sr^{l6}$ = *Df(3R)90D1; 90E1-2* and $sr^{l461}$ = *Df(3R)90C7-8; 90E1-2* (see Figure 4). Based on the data of SORSA (1988) we estimate that these deficiencies are larger than 70 kb and probably less than 300 kb. Hence, the essential gene described here and the *stripe* gene map within the 90D1; E1-2 cytological interval.

**Insertions at 90D are hypomorphic alleles:** Five enhancer detector strains with an insertion at 90D are homozygous viable. Four of these strains were isolated on the basis of their expression pattern, whereas one strain was isolated in a screen for mutations that cause reduced flight abilities. Complementation tests with these five strains, and the various deficiencies shown in Figure 4, and some of the homozygous lethal alleles indicate that these viable alleles can be subdivided in two groups (Table 1). Two insertions, named *cp1* and *cp2* (*cp* refers to a couch potato phenotype, see below), fail to complement or poorly complement most deficiencies and only partially complement the *l1* and *l2* insertions that cause lethality. Therefore, *cp1* and *cp2*, are hypomorphic viable alleles of the essential complementation group defined by the *l1-9* insertional alleles. The other three homozygous viable 90D insertions, *v1*, *v2* and *v3*, show no reduced viability in various complementation tests. The *cp1* and *cp2* alleles also allow us to order the different alleles in a progressive allelic series since they partially complement some homozygous lethal insertions: *l1–8* > *l9* > *cp1* = *cp2*. The complementation data indicate that none of the insertional alleles are null alleles since for example *cp1/l1* or *cp1/l2* flies have reduced viability but *cp1/deficiency* flies are sub-lethal and *l1/deficiency* or *l2/deficiency* flies are lethal (Tabel 1).

To define the embryonic mutant null phenotype it is necessary to recover amorph alleles. Hence, we performed a Δ2–3-transposase excision mutagenesis (BELLEN *et al.* 1989). About 500 $ry^{-}$ flies that lost part or all of the 90D *cp1* enhancer detector were screened, and two newly isolated mutants failed to complement *l1-2* as well as *Df(3R)P14*. These new alleles were named *lΔcp11* and *lΔcp12*. As shown in Table 1, the *lΔcp11* mutation fails to complement the lethality of all other alleles tested and thus may be an amorph allele. The other allele *lΔcp12* behaves as a relatively weak hypomorph in the various complementation tests. Based on the complementation data presented in Table 1 we reordered the alleles with decreasing strength in the following allelic series: $Df(3R)sr^{l461}$ = $Df(3R)sr^{l6}$ = *lΔcp11* > *l1–8* > *l9* > *lΔcp12* > *cp1* = *cp2*.

***cpo* is required for embryonic viability, and *cpo* mutant adults are couch potatos:** We anticipated that flies homozygous for hypomorphic alleles of *cpo* may show adult behavioral defects since a mutant was isolated in a screen for reduced flight ability. We also noticed that homozygous *cp1* adults recover more slowly after ether anaesthesia than the heterozygous balanced flies, indicating that several behavioral defects may be associated with these insertion chromosomes. Hence, we decided to perform a series of simple behavioral experiments with flies carrying independently isolated alleles from three different genetic screens. We selected four simple assays to measure phototaxis (BENZER 1967), geotaxis, flight response and recovery time after ether anaesthesia (see MATERIAL AND METHODS).

Table 2 summarizes the result of these behavioral tests. For each assay the results could most easily be subdivided into three groups: a high (+), an intermediate (+/−), and a low scoring (−) class (see MATERIALS AND METHODS). The data show that homozygous *cp1*, *cp2* and *v3* flies exhibit behavioral defects in all assays. In addition, the *l1* and *l2* insertional alleles fail to complement the *cp1* and *cp2* alleles in these four assays. However, although homozygous *v3* flies are behaviorally defective in the four assays, the *v3* insertion fully complements the *l1*, *l2* and *cp2* insertions. This suggests that the *v3* insertion complements mutations in the same gene, or that *v3* affects another nearby gene whose absence or reduction causes a similar phenotype. To determine if other mutations that are not associated with the insertions contribute

**TABLE 1**

**Complementation data for insertional alleles and deficiencies at cytological subdivision 90D**

| | *l9* | *l1* | *l2* | *cp1* | *cp2* | *v1* | *v2* | *v3* | *lΔcp11* | *lΔcp12* |
|---|---|---|---|---|---|---|---|---|---|---|
| *l9* | – | | | | | | | | | |
| *l1* | – | – | | | | | | | | |
| *l2* | – | – | – | | | | | | | |
| *cp1* | + | +/– | +/– | + | | | | | | |
| *cp2* | + | +/– | +/– | + | + | | | | | |
| *v1* | + | + | + | nt | + | + | | | | |
| *v2* | + | + | + | + | + | + | + | | | |
| *v3* | nt | + | + | + | + | + | nt | + | | |
| *lΔcp11* | – | – | – | – | – | + | + | + | – | |
| *lΔcp12* | +/– | +/– | +/– | + | + | + | + | + | – | – |
| *Df(3R)P14* | – | – | – | –/+ | nt | nt | nt | nt | – | – |
| *Df(3R)glBX10* | nt | – | – | – | – | + | + | + | – | – |
| *Df(3R)glBX6* | nt | – | – | –/+ | – | + | + | + | – | – |
| *Df(3R)DG4* | – | – | nt | –/+ | –/+ | + | + | nt | – | – |
| *Df(3R)sr*$^{l6}$ | – | – | – | –/+ | nt | nt | nt | nt | – | – |
| *Df(3R)sr*$^{l461}$ | – | – | – | –/+ | nt | nt | nt | nt | – | – |
| *Df(3R)glBX1* | + | + | + | + | + | nt | nt | nt | + | + |
| *sr*$^{1}$ | + | + | + | + | + | + | + | + | + | + |

*– = no transheterozygous progeny recovered; + = more than 50% of the expected transheterozygous progeny recovered; +/– = less than 50% but more than 10% of the expected transheterozygous progeny recovered; –/+ = less than 10% of the expected transheterozygous progeny recovered; nt = not tested.*

to the behavioral defects, the *cp1*, *v3* and *l1* insertions were excised using the Δ2–3-transposase mutagenesis scheme. At least 10 independent homozygous viable strains for each of these three insertion strains were recovered and further analyzed. These viable fly strains were tested in all the behavioral assays. For each original insertion at least two independent revertant strains were recovered which scored positive (+) in all assays. These observations indicate that these three insertions are responsible for the behavioral defects or for lethality in the case of the *l1* insertion. In summary, these data indicate that we have recovered many mutants in an essential gene and that some insertional alleles affect the function of a gene that is required for normal behavior.

Viable mutant flies (*e.g.*, *cp1/cp1*, *cp2/cp2*, *cp1/l1*, *cp2/l1*) show not only abnormal adult behavior but their development is also significantly delayed since these flies eclose at least 1 and usually 2 days after the balanced flies. In addition, while the flight assays may give the impression that *cpo* mutants are unable to fly, many are able to fly a limited distance when prodded. Finally, *cp1* and *cp2* flies groom most of the time while being tested in the behavioral assays. Based on the abnormal and hypoactive behavior of the mutant flies we have named the gene *couch potato* (*cpo*).

Most *cpo* alleles cause lethality late in embryonic development. For example, homozygous *cpo*$^{l1}$ and *cpo*$^{l\Delta cp11}$ first instar larvae fail to hatch and are sluggish when extracted manually. However, we did not observe any obvious defects or reduction in muscle size in any *cpo*$^{l\Delta cp11}$, *cpo*$^{l1}$ or *Df(3R)DG4* embryos using immunocytochemistry with a muscle-specific antibody MAb6D5 (data not shown). To screen for defects in the CNS and the PNS of *cpo* mutant strains, we immunocytochemically stained the nervous system of embryos homozygous for many different *cpo* alleles with anti-HRP (Jan and Jan 1982), anti-cut (Blochlinger *et al.* 1990) and monoclonal antibody 22C10 (Canal and Ferrus 1986). No obvious defects were observed in the nervous systems of embryos of strains carrying insertional alleles or *cpo*$^{l\Delta cp11}$. Thus, *cpo* is essential but we observe no obvious morphological defects in cuticle, muscles and nervous system.

**Enhancer detector insertions in *cpo* map to a 250-bp fragment:** Genomic DNA flanking three independently isolated insertions in or near *cpo* were isolated using the plasmid rescue technique (see Wilson *et al.* 1989). These rescued fragments, which map at cytological subdivision 90D1-6, were used to screen a lambda DASH library, and about 50 kb of contiguous sequences of genomic DNA in the 90D region were isolated (see Figure 5).

To identify the functionally important domains of *cpo*, we first mapped the positions of 13 enhancer detector insertions with Southern analyses. All the insertions mapped so far are located within a 250-bp genomic fragment. One insertion that causes homozygous lethality (*l9*) maps in the left half of this genomic fragment (Figure 5). The *l9* insertion behaves as a relatively weak hypomorph in complementation tests (*cpo*$^{l9}$ in Table 1). All other insertions map 150–250 bp to the right of the *l9* insertion (*l1–6, 8, v1, v2, v3, cp1* and *cp2*). The three insertions that are homozygous viable (v1-v3) map in a different orientation than all other insertions. The *v3* insertion causes a

TABLE 2

**Behavioral assays**

| | *l1* | *l2* | *cp1* | *cp2* | *v3* | *CS* | *TM3* | *ry* |
|---|---|---|---|---|---|---|---|---|
| Phototactic response | | | | | | | | |
| *cp1* | − | − | − | − | nt | + | +/− | + |
| *cp2* | − | − | − | − | + | + | +/− | nt |
| *v3* | + | + | nt | + | − | + | +/− | nt |
| *CS* | + | + | + | + | + | + | nt | nt |
| Flight response | | | | | | | | |
| *cp1* | − | − | − | nt | nt | + | +/− | + |
| *cp2* | − | − | nt | − | + | + | +/− | + |
| *v3* | nt | nt | nt | + | − | + | +/− | + |
| *CS* | + | + | + | + | + | + | + | nt |
| Geotactic response | | | | | | | | |
| *cp1* | − | − | − | nt | nt | + | +/− | +/− |
| *cp2* | − | − | nt | − | + | + | +/− | + |
| *v3* | + | +/− | nt | + | − | + | +/− | +/− |
| *CS* | + | + | + | + | + | + | nt | nt |
| Ether recovery | | | | | | | | |
| *cp1* | − | − | − | − | nt | +/− | +/− | −/+ |
| *cp2* | − | − | − | − | + | + | +/− | + |
| *v3* | + | + | nt | + | − | + | +/− | −/+ |
| *CS* | + | + | + | + | + | + | − | nt |

See MATERIAL AND METHODS for symbols.

cpo phenotype but complements the other *cpo* alleles, including the deficiencies. Six other insertions map approximately 40–60 bp to the left of the cluster of the three viables. They are homozygous lethal. Finally, the $cpo^{cp1}$ and $cpo^{cp2}$ insertions map close to the *v1–3* and the *l1–6* insertions. It should be noted that these mapping data are tentative since they are based on genomic Southern, and plasmid rescue data. Since none of these insertions seem to be null alleles of *cpo*, they are probably located in a regulatory region of the gene.

Genomic Southern analysis also revealed that $cpo^{l\Delta cp11}$ is a deletion which is possibly combined with a rearrangement of *P* element sequences (see Figure 5), whereas $cpo^{l\Delta cp12}$, is an internal deletion of the *cp1* enhancer detector (data not shown). Quantitative genomic Southern analysis showed that $Df(3R)sr^{l6}$ and $Df(3R)sr^{l461}$ lack the DNA corresponding to the entire genomic walk shown in Figure 5. This was also confirmed by *in situ* hybridization to polytene chromosomes carrying these deficiencies using various fragments from the genomic walk as probes (data not shown).

**The 90D insertions are in an intron of *cpo*:** A 5-kb *Not*I-*Hin*dIII plasmid rescued genomic fragment at the 3′ end of the $cpo^{cp1}$ insertion was previously shown to contain no repetitive DNA and to hybridize to a major 2.7–2.8-kb transcript (and possibly to other minor transcripts in the 1.6- and 4.4-kb range; $cpo^{cp1}$ corresponds to strain B52.1M3 in Figure 4 of WILSON *et al.* 1989; the fragment used for genomic Southern and Northern blots in this figure is the 5-kb *Not*I-*Hin*dIII fragment). This genomic fragment was used

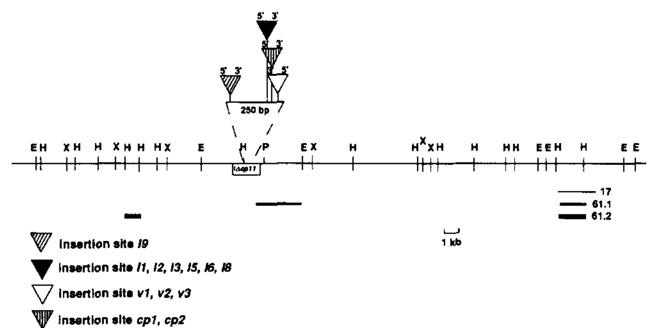


FIGURE 5.—Molecular map of *cpo* with the mapping position of the *P* element insertions and the hybridization sites of the three longest cDNAs. $cpo^{l\Delta cp11}$, the most severe allele, is a deficiency. The excision event has lead to the loss of a *Bam*HI site which is located about 200 bp to the left of the *cp1* insertion. The precise extent of the deficiency is unknown but restricted to the boxed region in the figure. The $cpo^{l1-8}$ are hypomorphic lethal alleles, and most map within 50 bp. The $cpo^{l9}$ allele is a weaker hypomorph than the $cpo^{l1-8}$ alleles and this insertion maps at a different position. Note that insertions that are homozygous viable (*v1*, *v2* and *v3*) are inserted in the opposite orientation than other insertions. These insertions behave differently in complementation tests than the *cp1* and *cp2* insertions which are inserted in the opposite orientation. The data indicate that the position of the insertion, and possibly the orientation and size of the insertion may play a role in the mutant phenotype. E = *Eco*RI, H = *Hin*dIII and X = *Xba*I.

to screen an embryonic lambda gt10 cDNA library (provided by L. KAUVAR). Two cDNAs of about 1.6 and 1.8 kb were isolated, and these were used to screen a size selected 9–12-hr-old embryonic cDNA library (ZINN, MCALLISTER and GOODMAN 1988). The three longest cDNAs, 17, 61.1 and 61.2, out of 62 positives were selected for further work. These cDNAs are 3.0, 3.6 and 2.5 kb long, respectively. Southern analysis shows that each of these cDNAs is

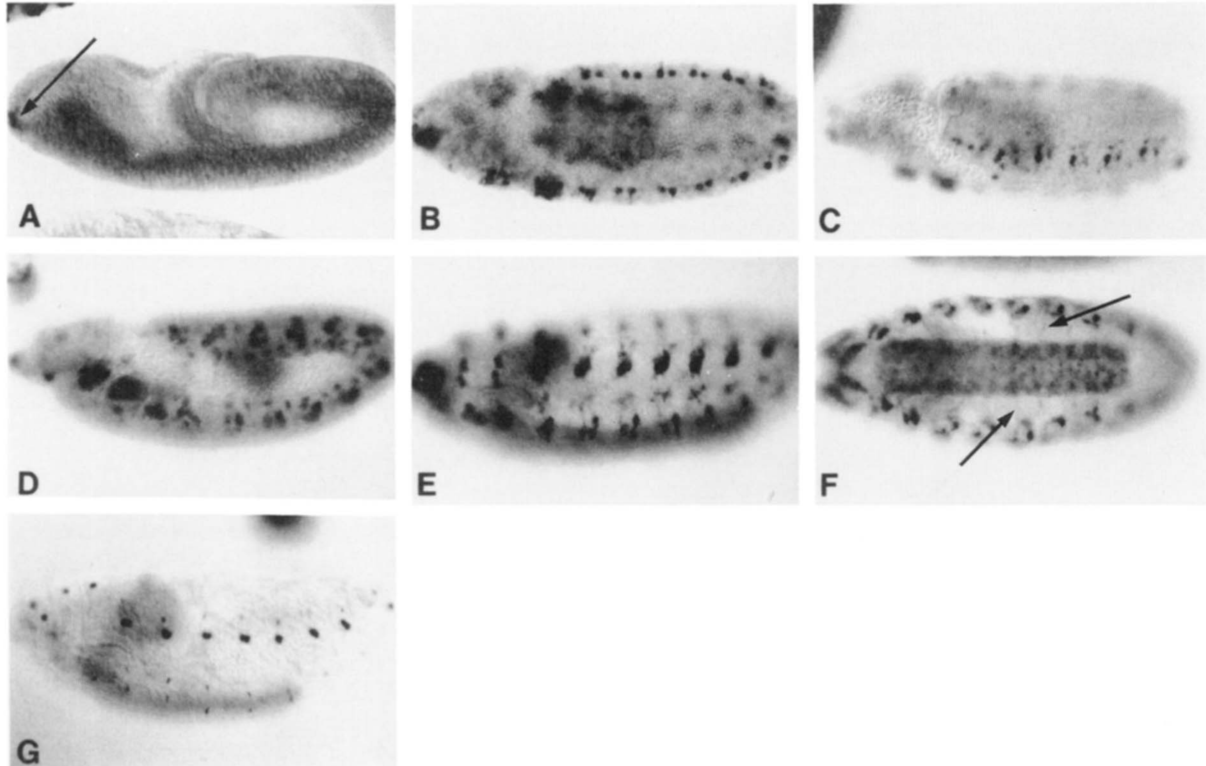


FIGURE 6.—Embryonic *in situ* expression pattern of *cpo*. Compare this figure with Figure 1. A, Stage 8; note expression at most anterior end of embryo (see arrow). B, Stage late 10 to early 11, dorsoventral view. C, Stage mid 11, dorsoventral view. D, Stage late 11 to early 12, lateral view. E, Stage 16, lateral view. F, Stage 16, ventral view: note the label along the nerves (see arrows). G, Stage 17, lateral view of an embryo that is homozygous for *cpo*[11]. *cpo* expression is restricted to the chordotonal neurons.

derived from a message with a different splicing pattern. Since the 61.2 cDNAs strongly hybridizes to genomic fragments on both sides of the insertions we conclude that the enhancer detectors are most likely inserted in an intron of the transcription unit of *cpo*. Genomic Southern analyses, Northern analysis and reverse Northern analysis indicate that the three cDNAs contain highly repetitive DNA which is confined to a 2.2-kb *Hin*dIII fragment shared by the three cDNAs (see Figure 5). Reverse Northern experiments covering the entire walk using 9–12-hr-old poly($A^+$) RNA also revealed that there are no other transcripts detected within the introns shown in Figure 5 (data not shown).

As shown in Figure 6, *in situ* hybridization experiments to whole mount embryos with the three digoxigenin labeled cDNAs show that the transcripts are expressed in the SMC of the PNS as well as in the differentiated cells of the PNS (Figure 6, B and C). Some cell clusters, for example cells in the lateral cluster, label more intensely than others (Figure 6E). The cytoplasm of many neurons is weakly labeled, and many axon bundles or nerves are labeled, especially in the ventral part of the embryo where the peripheral nerves connect with the CNS (Figure 6F). It is not clear whether transcripts in neurons contribute to the labeling of the axons because glial cells that are positioned along the peripheral nerves also express *cpo*. The glial cells form a sheath around the peripheral nerves, and this sheath is most easily seen in the vicinity of the CNS. The cytoplasm of these glial cells as well as their projections are clearly labeled, masking possible staining in axons. In some embryos we have been able to identify strong staining in cells located on the dorsal side and a pair of cells on the ventral side of the CNS. These cells seem to correspond in location to the cells that express the β-galactosidase enzyme in the insertion strains and are most likely glial cells.

A comparison of the embryonic β-galactosidase staining of the *cpo* enhancer detector strains with the expression pattern of the *cpo* mRNAs shows that the enhancer detectors quite faithfully reproduce the expression pattern of the transcripts. The only obvious difference in the two patterns shown in Figure 1 and Figure 6 can be attributed to the nuclear localization of the β-galactosidase staining (BELLEN *et al.* 1989; WILSON *et al.* 1989) and the predominantly cytoplasmic staining of the *cpo* transcripts. During the early developmental stages both patterns are very similar. For example, stage 7–8 embryos express the *lacZ* and the transcript in a few cells at the most anterior end of the embryo. In stage 9–10 embryos a few cells in in each presumptive segment express the

transcript at about 4:20–4:40 hr of development, about 0.5 hr earlier than the β-galactosidase expression in these cells. In addition, when both types of staining are compared, subtle differences can be seen in the intensity with which some cells or cell clusters of the PNS are labeled. The *lacZ* expression staining intensity is similar in most cells of late embryos whereas the transcript seems to be more abundant in specific cells, especially the chordotonal organs. The major difference between the two expression patterns is that faint staining is observed in most of the CNS in the *in situ* hybridizations. However, this label may be nonspecific since homozygous $Df(3R)sr^{l6}$ embryos also stain in the CNS.

**Expression of *cpo* is altered in some mutants:** Mutant embryos that lack part or most of the *cpo* gene should show alterations in *cpo* expression. We compared expression of *cpo* in mutant ($cpo^X/cpo^X$) and wild type ($cpo^X/+$, or $+/+$) embryos using whole mount *in situ* hybridizations. Expression of *cpo* is clearly altered in embryos homozygous for deficiencies of *cpo*. Embryos homozygous for $Df(3R)sr^{l6}$ are embryonic lethal and lack most or all the sequences to which the *cpo* cDNAs hybridize. About one quarter of the embryos of a $Df(3R)sr^{l6}$/TM1 strain show no labeling except in the CNS. Homozygous lethal $cpo^{l1}$ embryos or larvae show no obvious defects in the PNS or the CNS but about ¼ of the embryos of the balanced strains exhibit a very severe reduction of the amount of label in cells of the PNS and in older embryos (stage 14–17) label is seen in the chordotonal neurons only (compare Figure 6G with Figure 6E). Similar observations were made for the embryos of $cpo^{l\Delta cp11}/TM3$ flies, indicating that 90D insertional mutations and a deficiency at the insertion site affect the level of the *cpo* mRNAs and the spatial and temporal expression of *cpo*.

## DISCUSSION

In this work we describe the genetic and phenotypic characterization along with the preliminary molecular analysis of a newly identified gene named *couch potato* (*cpo*). Insertions in *cpo* have been isolated in several independent enhancer detector screens because expression of the *lacZ* reporter gene is essentially confined to the SMC and the more differentiated cells of the PNS during embryonic development. These insertion strains are interesting because few other enhancer detector strains at positions other than 90D label the sensory mother cells and their progeny. In addition, although most sensory organs are dispensible for viability, a number of *cpo* insertional mutations were found to be embryonic lethal suggesting that the gene plays an important role in the development or function of the PNS.

The observation that *cpo* is essential raises an important question: In which tissue is *cpo* required? A detailed analysis of the *lacZ* expression pattern in the enhancer detector strains showed that *lacZ* is expressed in many glial cells of the CNS and PNS and at low levels in some cells of the midgut. *In situ* hybridizations to whole mount embryos with *cpo* cDNAs indicate that *cpo* expression is confined to PNS and glia, and possibly to the CNS. However, we have been unable to establish if expression in the CNS is significantly higher than background. We also found no convincing evidence that *cpo* is expressed in the midgut. Since no defects were observed in muscles, PNS or CNS of amorphic mutant embryos it is possible that *cpo* is required in the embryonic nervous system for an essential behavioral, biochemical, or physiological function without affecting the morphology of CNS or PNS. If *cpo* is required in the PNS only for viability, then one would have to conclude that the chordotonal organs are essential since some viable mutations in the *scute-achaete* complex cause loss of all PNS organs except the chordotonals. Future molecular and genetic experiments will address these questions.

All insertional alleles at 90D mapped thus far are confined to a 250-bp interval. These sequences appear to be in an intron of one *cpo* transcript, and 5′ of the start of another cDNA (data not shown). Several observations indicate that these and neighboring sequences play an important role in regulation of *cpo* transcription. First, all insertional mutations in 90D tested either cause lethality or behavioral defects (the *v1* and *v2* insertional strains have not been tested yet). Second, an overall decrease in level of *cpo* transcript in the PNS is observed in some insertion strains, and in homozygous *l1* embryos the transcript is confined to the chordotonal neurons of stage 14–17 embryos only. Third, a small deletion at the insertion site leads to the same expression pattern as the one observed in homozygous *l1* embryos. We therefore conclude that these sequences are essential for proper expression of *cpo* in the PNS and the glial cells and that they contain key regulatory sequences.

A number of *cpo* alleles cause severe behavioral defects in adults. Some of these behavioral abnormalities may be enhanced by other uncharacterized mutations but results of various behavioral assays with revertants clearly indicate that *cpo* is required for normal adult behavior. However, the behavioral defects may be indicative of general sickness rather than specific behavioral deficits. An analysis of the *lacZ* pattern in the various 90D enhancer detector strains suggests that *cpo* is expressed in the CNS, PNS and salivary glands of third instar larvae and adults but not in most other tissues. We therefore conclude that the *cpo* gene is probably required in the mature nervous system for normal adult behavior.

Complementation and behavioral tests showed that

the *v3* insertion causes behavioral defects but that these defects are not observed in *trans*-heterozygous flies that carry a *v3* insertion and another *cpo* insertion that affects behavior or causes lethality. Since excision of *v3* allowed us to recover several strains that exhibit wild type behavior, we conclude that the *v3* insertion causes a *cpo* phenotype, and that other uncharacterized mutations are unlikely to play a role in the phenotype. It is possible that *v3* may affect another gene near *cpo* which plays a similar role as *cpo*, but this seems unlikely since *v3* is inserted next to the insertions that cause lethality and those that affect behavior. We therefore conclude that the *v3* insertion complements other insertions that cause a cpo phenotype. This intragenic complementation may be due to transvection-like phenomena since insertions may disrupt somatic pairing of the chromosomes in *trans*-heterozygous flies.

Mutations in *cpo* and *stripe* fail to complement all tested deficiencies in cytological subdivisions 90D/E. Since mutants for either gene fail to fly, and since preliminary evidence suggests that *stripe* is required in the nervous system (COSTELLO and WYMAN 1986; DE LA POMPA, GARCIA and FERRUS 1989), we investigated the possible relationship between these two genes in greater detail. Based on the following observations we believe that *cpo* and *stripe* are different genes that map in close proximity to each other: first, mutations in both genes complement each other for the typical stripe phenotype, *i.e.*, $sr^1/cpo$ flies do not show a dark thoracic stripe; second, none of the behavioral defects observed in *cpo* mutants is observed in *trans*-heterozygous *sr/cpo* flies; third, genomic fragments adjacent to enhancer detector insertions in the *stripe* gene are not contained in the 50 kb genomic walk shown in Figure 4; fourth, *in situ* hybridization experiments to polytene chromosomes with these sequences and those from the 90D walk revealed that sequences of both walks hybridize respectively to 90E1-2 (*stripe*) and 90D2-6 (*cpo*), in agreement with the mapping positions of the respective enhancer detector insertions; fifth, the β-galactosidase expression pattern of enhancer detector strains inserted next to or in *stripe* show a very different expression pattern (data not shown). These observations provide compelling evidence that both genes are different. However, some muscle defects have been observed in some *trans*-heterozygous *stripe/cpo* flies, suggesting that both genes may somehow interact (data not shown).

In conclusion, we have shown that *cpo* is required for embryonic viability and for normal adult behavior. Its expression pattern indicates that it may play an important role in the PNS and/or glia. However, absence of the gene product does not cause any obvious morphological defects in these cells indicating that *cpo* may play an unknown biochemical or physiological role. The *cpo* gene is possibly one of the hottest spot for *P* element-enhancer detector insertions and genetic evidence indicates that the genomic region in which the *P* elements have inserted is a key regulatory region of the gene. Further molecular and genetic analyses are in progress to determine what the role of *cpo* is in the nervous system.

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